1950

Host-parasite relationship between Cynaeus angustus Lec. and Nosema cynaea Sp. Nov.

Jack Louis Krall
Iowa State College

HOST-PARASITE RELATIONSHIP BETWEEN *CYNAEUS ANGUSTUS* LEC. AND *NOSEMA CYNAEA* SP. NOV.

by

Jack Louis Krall

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1950

TABLE OF CONTENTS

INTRODUCTION. 1

REVIEW OF LITERATURE 4

Taxonomy 4
Stages of Development in Microsporidia 7

Spore 7
Vegetative stages 13

Host-parasite Relations. 17

Methods of infection. 17
Results of infection. 18
Specificity of Microsporidia. 22
Hosts 22

TAXONOMY OF THE PARASITE. 24

EXPERIMENTAL. 27

Materials and Methods. 27
Description of Life Stages of the Parasite 36

Amoebula. 36
Planont 37
Meront. 37
Sporont and sporoblast. 38
Spore 38

Development of the Parasite and Its Effect on the Host 49

Spore germination 49
Length of life cycle. 56
Mode of infection and dissemination of spores. 58
Macroscopic symptoms of disease 60
Reliability of macroscopic symptoms in diagnosis 63
Time required for spots to appear in larvae . . 63
Effect of parasitism on larval weight 66
Infection early and late in larval life 69
Life study of diseased and healthy larvae . . . 70
Microscopic observations of tissues 72

Parasitism and host populations. 73
Miscellaneous observations 74

SUMMARY AND CONCLUSIONS. 77

GLOSSARY 80

LITERATURE CITED 82

ACKNOWLEDGMENTS. 88

INTRODUCTION

The insect, Cynaeus angustus Lec., family Tenebrionidae, order Coleoptera, was described in 1852 by Le Conte (37) from specimens collected in the Colorado Desert of California. He first named it Platydema angustum Lec. but 10 years later the same author (38) proposed the generic name Cynaeus Lec. and designated Platydema angustum Lec. as the type species.

Until 1939, when Hatch (16) reported the beetle in a flour mill in Western Washington, the information regarding this insect was entirely of a taxonomic nature. Hatch (17), in 1940, added to our knowledge of the insect's habits when he reported that Dr. F. E. Blaisdell had observed it about the base of yucca plants in California. In the same year, Decker (5) reported the insect from Iowa in shelled corn and, at that time, the beetle was brought to the attention of the present author.

The writer began his graduate study in September, 1940, at Iowa State College and, at the suggestion of Dr. Carl J. Drake and with his guidance, undertook to study the biology of this insect. In the early part of 1942, it was observed that some of the larvae in cultures developed blackened areas clearly visible through the integument. Since gross anatomical studies did not reveal the nature or cause of these abnormalities, serial sections were prepared and

examined. Some tissues were found to contain spores which Dr. E. R. Becker, of Iowa State College, identified as those of a microsporidian parasite.

Following this observation, and to determine what effect the parasite had on the life of its host, a number of diseased larvae were segregated individually in vials, and it was soon apparent that infection by the parasite was fatal to the insect. The study of the host-parasite relationship was considered to be beyond the scope of the original problem and, consequently, only miscellaneous observations were made. The results of the biological study on the insect and a few observations on the parasite were published in 1946 by Krall and Decker (22).

In January, 1946, the author returned to Iowa State College to continue his graduate work, and in considering a thesis problem, the host-parasite relationship of these two organisms seemed worthy of attention. The insect is a pest of stored grains, particularly corn, and as such is economically important. Consequently, any information obtained on its enemies would be a worthwhile contribution to a better understanding of the biological relations of these two organisms.

The purpose of the present study was to obtain as much information as possible on the life cycle of the parasite and to determine what effect its presence had on the host.

This involved not only a study of the stages in the life of the parasite, but also its transmission from host to host, the insect tissues attacked, and the significance of the protozoan in the biological control of the insect.

REVIEW OF LITERATURE

Taxonomy

A monograph of the Microsporidia was published in 1924 by Kudo (31) in which he reviewed the morphology and development of these organisms, discussed the relationships between the parasites and their hosts, and gave a complete taxonomic survey of the group. This taxonomic survey included not only those species definitely established to be Microsporidia but also those the identity of which was doubtful. In view of the nature of the monograph, the present author decided to summarize those pertinent facts contained in Kudo's paper and to append any subsequently reported which were considered related to the present problem.

The first microsporidian, although not known as such at the time, came to the attention of the scientific world about 1850 when a disease of silkworms, known as pébrine, reached epidemic proportions and threatened to wipe out the silkworms in the sericultural countries of Europe. As the production of silk was a major industry in Southern Europe, the disease came to the attention of Pasteur.

In 1857 Nägeli (46) gave the name Nosema bombycis to the causative organism, and in 1882 Balbiani (2) proposed that this organism and all other related forms be named Microsporidia and placed in the class Sporozoa of the phylum Protozoa. The interest in N. bombycis led to the discovery

of many other species in this group parasitic on numerous invertebrates and some of the lower vertebrates.

Through the years, various systems of classification were proposed for this group of animals. Thélohan (55) in 1892 and Gurley (14) in 1893, independently proposed a system of classification based upon the mode of spore formation. Stempell (53) in 1909 came to the conclusion that an adequate system of classification must be based upon the form and development of the vegetative stages, the number of spores produced by a sporont, and the form of the spores. In 1922, Léger and Hesse (44) proposed a system based entirely upon the form and structure of the spores. Kudo (31) believed that the system proposed by Léger and Hesse was best suited to the present knowledge of the Microsporidia. His belief was based on the fact that the spore is the most conspicuous stage in the life cycle of a microsporidian and, in any one species, exhibits less variation in form than any of the other stages concerned in the life cycle. He also contended that not only the form, but the structure of the spore can be used for the separation of species.

The system of classification based on the form, structure and the number of spores produced by a sporont was proposed by Kudo (31, p. 65-69) and is quoted, in part, below:

> Order Microsporidia Balbiani 1882
> Intracellular parasites of typically invertebrates.
> Multiplication by schizogonic divisions.

Sporont develops into one to numerous spores. The minute spore is covered with a resistant membrane, possesses a sporoplasm and one, or rarely two, comparatively long filaments which are coiled in a polar capsule that is usually obscure in the fresh state.

Suborder Monocnidea Léger et Hesse 1922
Microsporidia, the spore of which is provided with one polar filament that is typically coiled in a polar capsule.

Family Nosematidae Labbé 1899
Spores oval, ovoid or pyriform. If subcylindrical, length is less than 4 times the breadth.

Genus Nosema Nägeli 1857 emend. Pérez 1905. . .
Each sporont develops into a single spore. Type species: Nosema bombycis Nägeli. . . . . .

Genus Glugea Thélohan 1891 emend. Weissenberg 1913. . .
Each sporont develops into two spores. Host cells become enormously hypertrophied, forming the so-called Glugea-cysts. Type species: Glugea anomala(Moniez) Gurley. . . . . .

Genus Perezia Léger et Duboscq 1909. . .
Each sporont forms two spores. Host cell is not hypertrophied as in the last mentioned genus. Type species: Perezia lankesteriae Léger et Duboscq.

Genus Gurleya Doflein 1898. . .
Each sporont produces four sporoblasts and ultimately develops into four spores. Type species: Gurleya tetraspora Doflein.

Genus Thelohania Henneguy 1892. . .
Each sporont develops into eight sporoblasts and ultimately into eight spores. The sporont membrane may degenerate at different times of development. Type species (proposed by Gurley): Thelohania giardi Henneguy.

Genus Stempellia Léger et Hesse 1910. . .
Each sporont develops into one, two, four or eight sporoblasts and ultimately into one, two, four or eight spores. Type species: Stempellia mutabilis Léger et Hesse.

Genus Duboscqia Pérez 1908 emend. . . .
Each sporont develops into 16 sporoblasts and ultimately into 16 spores. Type (and only) species: Duboscqia legeri Pérez. . . . . .

Genus Plistophora Gurley 1893. . .
Each sporont develops into many (more than 16) spores. Type species: Plistophora typicalis Gurley. . . . .
Family Cocconemidae Léger et Hesse 1922
Spores spherical or subspherical. . . . .
Family Mrazekidae Léger et Hesse 1922
Spores tubular or highly cylindrical (length is greater than 5 times the breadth). . . . .
Suborder Dicnidea Léger et Hesse 1922
Spore with two polar capsules, one at each end, containing a polar filament.
Family Telomyxidae Léger et Hesse 1910
With the characters of the suborder. . . . .

Stages of Development in Microsporidia

Spore

According to Kudo (31), the role of any stage involved in the development of a microsporidian cannot be minimized. The spores may be considered of the utmost importance biologically and taxonomically. Their biologic importance is concerned with the transmission of infection to new hosts and their ability to withstand adverse conditions; while from a taxonomic standpoint, an organism can neither be positively identified as a microsporidian, nor can it be differentiated from other species in the group without this stage.

The spores of different genera have been found to exhibit a considerable diversity of form. According to Kudo (31), the usual form of the spores was "oval", "ovoidal", "ovocylindrical", or "pyriform" with all the intermediate categories; and in some genera, the spores were "spherical",

"reniform", "tubular", "bacilliform", "crescent-shaped", "spiral", or "comma-shaped" (Fig. 1).

According to Kudo (31), the spores of any one species were generally more or less uniform in size, with the majority of species having spores 3 to 8 microns in length. In certain species a conspicuous polymorphism existed, as Kudo (34) found in Stempellia magna. The extremes of size variation are exemplified by Nosema pulvis with spores 1.25 x 1 microns as given by Pérez (50); and by Mrazekia argoisi with spores 17 to 23 x 3.5 microns as recorded by Léger and Hesse (41).

The spore membrane in most species was described as a refractive, smooth structure of uniform thickness and composed of one piece, Kudo (31). In Thelohania giardi, Thélohan (56) noticed longitudinal striations. Kudo (32) maintained that the spore membrane of T. opacita was bi-valved; and Goodrich (12) noted three tail-like extensions of the spore membrane in T. octospora.

The chemical nature of the spore membrane was not known until Kudo (26, p. 65) came to the conclusion that, "The spore membrane of Nosema bombycis and Nosema apis behaves very much like chitin under the influence of mineral acids, ... "

A great deal of controversy existed in the past, and has continued to the present time, regarding the internal details of the spores. This was true even in those cases in which different men studied the same species. These differences

of opinion Kudo (31) attributed to the minuteness of most spores, the limitations of optical apparatus and the difficulties encountered in obtaining penetration of the spore membrane by stains. Kudo (31, p. 20) states, "it is clear that the microsporidian spore is composed of three parts in all the well studied forms; i.e., the spore membrane, a sporoplasm and a polar filament, and that there are diverse opinions as to the finer structure of the sporoplasm such as its location and nucleus and the presence of a polar capsule." Differences of opinion as to the manner in which the filament was coiled in the spore have continued to the present time. Fantham and Porter maintained that in N. apis the filament passed through the sporoplasm and was coiled in the posterior vacuole (Fig. 2; G). Kudo with the same species, maintained that the filament was doubly coiled inside a polar capsule which occupied the anterior two-thirds of the spore (Fig. 2; F).

The polar filament, a characteristic of the Microsporidia, was first described by Thélohan (55) in 1892. In some species the filament is extremely long, fine and of uniform thickness throughout, while in others the basal portion of the filament was thickened (Fig. 2; A). It may be variously coiled in the capsule. It was doubly coiled as Kudo observed it in N. apis (Fig. 2; F), singly coiled (Fig. 2; B, C, E, G), coiled back on the basal portion which extended along the longitudinal axis of the spore (Fig. 2; A, D), or

Figure 1

A. Cocconema microccus after Léger and Hesse (43); "spherical".

B. Thelohania rotunda after Kudo (33); "oval".

C. Nosema bombycis after Kudo (23); "oval".

D. Glugea anomala after Weissenberg (58); "ovoidal".

E. Telomyxa glugeiformis after Léger and Hesse (40); "ovoidal".

F. Plistophora macrospora after Léger and Hesse (42); "ovoidal".

G. Glugea acuta after Thélohan (56); "pyriform".

H. Nosema cyclopis after Kudo (25); "pyriform".

I. Thelohania giardi after Mercier (45); "pyriform".

J. Gurleya francottei after Léger and Duboscq (39); "pyriform".

K. Stempellia magna after Kudo (34); "pyriform".

L. Thelohania reniformis after Kudo and Hetherington (36); "reniform".

M. Octosporea muscae-domesticae after Flu (9); "crescent-shaped".

N. Mrazekia bacilliformis after Léger and Hesse (44); "bacilliform".

O. Nosema marionis after Thélohan (56); "ovocylindrical".

P. Mrazekia mrazeki after Hesse (19); "tubular".

Q. M. argoisi after Léger and Hesse (41); "tubular".

R. Spironema octospora after Léger and Hesse (44); "spiral".

S. Toxonema vibrio after Léger and Hesse (44); "comma-shaped".

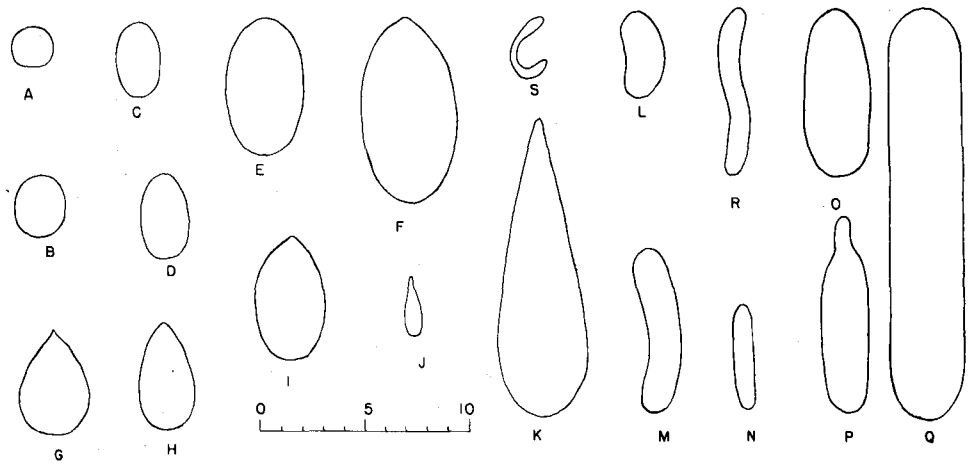

Fig. 1. Spore form of various species of Microsporidia. From Kudo (31). Scale in microns.

longitudinally coiled in the capsule as Ishiwata (20) observed it in a Nosema sp.

The function of the filament has not been satisfactorily explained. An hypothesis by Fantham and Porter (7) attributed to it the function of bringing the spore into close contact with the gut epithelium when the tip of the filament was anchored to this tissue. Korke (21) proposed that it served to advance the sporoplasm to an area distant from the spore.

The chemical nature of the polar filament has not been definitely established. Kudo (26) came to the conclusion that it was a mixture of nuclear material and some substance differentiated in the polar capsule. Stempell (53) advanced the hypothesis that the filaments were tubular. Strickland (54, p. 74) states, "this filament must, from the manner in which it is ejected, consist of a hollow tube which has to be entirely everted during its emergence from the spore!" However, he did not describe the manner in which the filament was ejected. According to Kudo (31), whether the filament is solid or a hollow tube has not been determined.

A polar capsule in the spore was described by Mercier for Thelohania giardi (Fig. 2; B); by Kudo for Stempellia magna and Nosema apis (Fig. 2; C, F); and by Stempell for N. bombycis (Fig. 2; D). Léger and Hesse did not mention a polar capsule for Mrazekia argoisi (Fig. 2; A); nor did Fantham and Porter for Nosema apis (Fig. 2; G).

The location and nucleation of the sporoplasm have been found to vary in different species and, by different authors, in the same species. Léger and Hesse in Mrazekia argoisi described the sporoplasm as an oval, bi-nucleated structure in the posterior part of the spore (Fig. 2; A). Mercier in Thelohania giardi described it as a band-shaped, 4-nucleated structure (Fig. 2; B). Kudo for Stempellia magna described it as a uni-nucleated mass of protoplasm in the posterior part of the spore (Fig. 2; C). Stempell for Nosema bombycis described the sporoplasm as a band-shaped structure in the anterior part of the spore; at first bi-nucleated and later 4-nucleated (Fig. 2; D). Kudo (23) for the same species believed that the sporoplasm was bi-nucleated. The sporoplasm of N. apis was described by Fantham and Porter as a band-shaped, bi-nucleated structure (Fig. 2; G) and by Kudo as a uni-nucleated, rounded mass in the posterior part of the spore (Fig. 2; F).

Vegetative stages

Differences of opinion have also existed in the descriptions of the vegetative stages and their development. To illustrate one version, the development of N. bombycis as worked out by Stempell (53) is included as figure 3 and summarized as follows:

When a spore of N. bombycis is ingested by a silkworm rapid changes take place. The two sporoplasm nuclei divide

Figure 2

A. Mrazekia argoisi after Léger and Hesse (41); showing thickened basal portion of polar filament.

B. Thelohania giardi after Mercier (45); showing band-shaped sporoplasm.

C. Stempellia magna after Kudo (34); showing sac-like polar capsule.

D. Nosema bombycis after Stempell (53).

E. Plistophora macrospora after Léger and Hesse (42); showing polar capsule lining the spore membrane.

F. Nosema apis after Kudo (24); showing doubly coiled polar filament.

G. Nosema apis after Fantham and Porter (7).

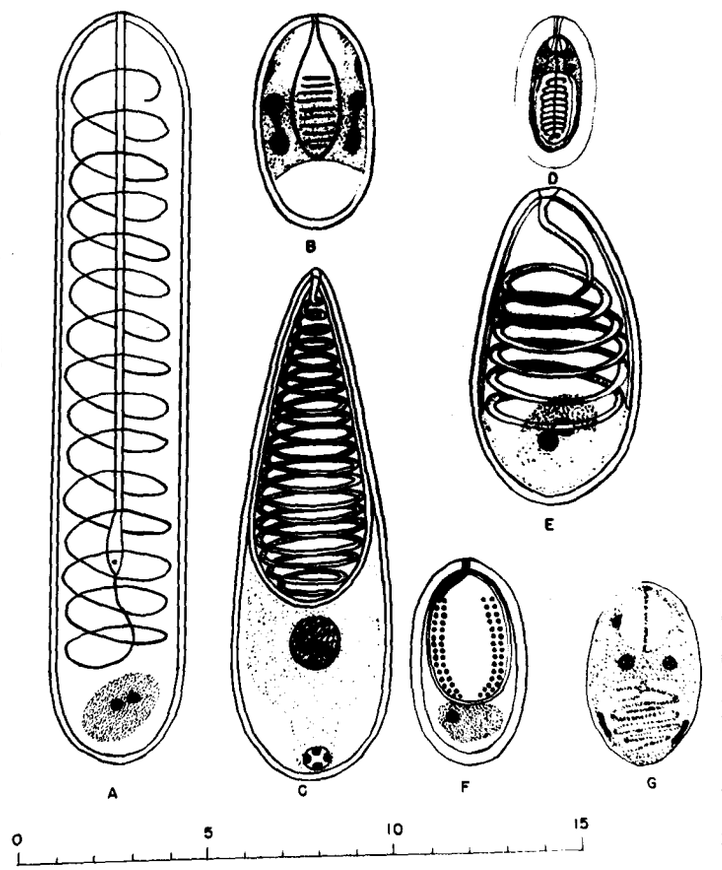

Fig. 2. Spores of species of Microsporidia. From Kudo (31). Scale in microns.

into four of equal size and the polar filament is extruded (v) and becomes detached. The sporoplasm, leaving two nuclei behind, escapes through the foramen as a bi-nucleated amoebula (w) which transforms, by the fusion of the nuclei, into a planont (a) capable of amoeboid movement. The planonts multiply by binary fission (b) and through their movements invade various host tissue cells. The intracellular stages, called meronts (e, l), are incapable of movement and obtain nourishment from the cytoplasm of the host cell by osmosis. Multiplication by binary fission (m-p) and by budding or multiple fission (f-k) also occurs during this stage. As a result, the host cell becomes filled with meronts, each of which, as space and food are exhausted, transforms into an ovoid sporont. The sporont nucleus divides to form two shell-nuclei, a nucleus for the polar capsule and two nuclei for the sporoplasm. The spore membrane forms and vacuoles appear at the poles while the sporoplasm assumes a band-shape in the anterior end of the spore, and the polar capsule with its filament becomes developed (q-s).

Trager (57), with N. bombycis, substantiated both the presence of the bi-nucleated amoebula as a sphere attached to the distal end of the extruded filament, and of the planont as the intercellular stage. Kudo (23), however, was not able to recognize the planont stage. Fantham and Porter (7) with N. apis described the same stages in the development of this

species as did Stempell with N. bombycis. Hertig (18) in his study of N. apis was not able to make out the planont stage nor did he observe the large multinucleated meronts described by Fantham and Porter.

The entire process of the emergence of a living amoebula was not observed by Stempell (53) or Kudo (23) in N. bombycis; by Fantham and Porter (7) in N. apis; nor by Kudo (34) in Stempellia magna. However, these workers believed that the amoebula escaped through the foramen in the membrane after the filament was detached.

Host-parasite Relations

Methods of infection

The infection of new hosts by most species of Microsporidia occurred when the spores were ingested. This was shown to be the case with Nosema bombycis by Stempell (53) and Kudo (23); for N. apis by Fantham and Porter (7); for Thelohania opacita by Kudo (32); and for Stempellia magna by Kudo (34). Auto-infection was believed to occur in Nosema bombycis by Kudo (23), and in N. apis by Fantham and Porter (7). In addition to the above, germinative infection was established for N. bombycis by Pasteur (48) in 1870. Since that time, spores of Octosporea muscae-domesticae have been found in the eggs of Musca domestica by Flu (9), and spores of an unidentified microsporidian by Nicholson (47) in the eggs of Anopheles maculipennis.

Results of infection

Some species of parasites caused color changes in the hosts. The host changed from a translucent appearance to one of opacity or dark brown, black or red areas formed in the infected tissues and were seen through the integument. Goodrich (13) maintained that the brown spots which appeared in the crustaceans Gammarus chevreuxi and G. pulex when infected by Thelohania mulleri or a Nosema sp. were caused when blood or hypodermal cells surrounded a mass of spores released from ruptured host cells and secreted a substance around the spores. This material was yellow brown in color and chitinoid in appearance. She found, however, that this material when tested for chitin reacted negatively.

Certain microsporidial infections prevented growth of the host or caused distortions. Silkworms, heavily infected by Nosema bombycis, remained small; and larvae of Culex territans, heavily infected by Stempellia magna, were smaller than normal according to Kudo (23, 28). He also found that during the later stages of the disease these hosts became sluggish, apparently because the enormous number of spores interfered with the functioning of the muscles. The host became inactive when the seat of infection was the muscle tissue as Kudo (25) found to be the case when Cyclops albidus was parasitized by Nosema infirmum.

Figure 3

I. Extracellular stages.

II. Intracellular stages.

a-c. Planonts.

m-p. Meronts in binary fission.

f-k. Meronts in multiple fission and budding.

q-s. Stages in sporulation.

t,u. Spores in midgut of a new host.

v. Extrusion of the polar filament.

w. Amoebula leaving the spore.

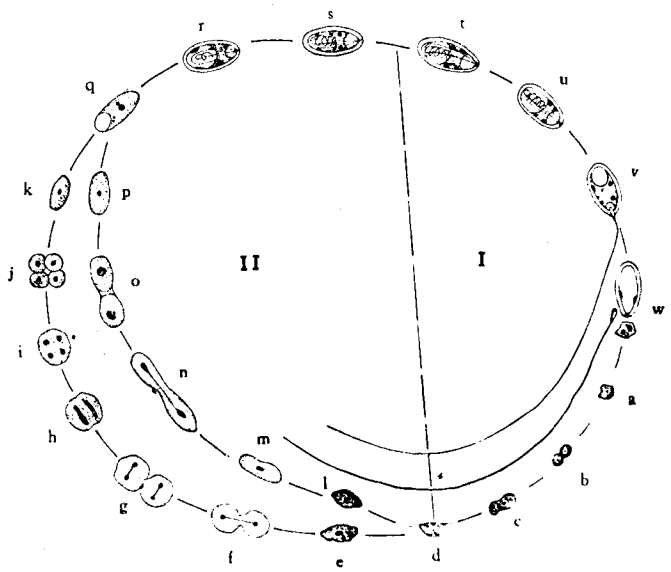

Fig. 3. The life cycle of Nosema bombycis. After Stempell (53). From Kudo (31).

Nuclear hypertrophy of the host cells was observed to occur in parasitized larvae of Culex pipiens by Kudo (28) and by the same author (29) in Baetis pygmata Hagen (?) attacked by Thelohania baetica. Hertig (18) observed an increased proliferation of the epithelial cells of the mid-gut of bees infected by Nosema apis. The cytoplasm of the invaded cells became greatly distended due to the presence of many parasites.

The presence of a microsporidian in a female host accounted for the lack of egg-laying females, although the ovaries were not the seat of infection. This was evidently the case with Velia currens attacked by N. veliae. Poisson (51, p. 60) states, "Chez les femelles de Velia parasitées les ovules dégénèrent fréquemment et chez beaucoup de mâles, les testicules demeurent rudimentaires." Simuliid larvae attacked by species of Thelohania were found by Strickland (54) to lack reproductive organs.

Those Microsporidia known to cause the death of the host when heavy infections occur are the species which have been most thoroughly studied. This was the case with silkworms attacked by Nosema bombycis as reported by Pasteur (48) and Kudo (23). Fantham and Porter (7) and Hertig (18) attributed the death of honey bees to infection by N. apis; and Strickland (54) observed that Simuliid larvae, infected by species of Thelohania, died before transforming to the pupal stage.

Specificity of Microsporidia

Kudo (31, p. 40) states, "There seems to be a definite relation between a microsporidian parasite and its host; and in numerous cases the microsporidian invades only a specific tissue cell of the host animal." In the silkworm, however, the parasite invaded all the tissues. An excellent example of host specificity was observed by Kudo (30, 33) in his studies of mosquito larvae. The species Thelohania legeri, T. obesa and Nosema anophelis were exclusively parasitic on mosquitoes of the genus Anopheles, in France and the United States; while Thelohania opacita, T. rotunda, T. minuta, and Stempellia magna attacked only the larvae of the genus Culex. Although Kudo found larvae of the two host genera living in the same small bodies of water, not a single case of mixed infection occurred. Kudo believes strongly in the host specificity of the Microsporidia. In describing Nosema termitis, he (35, p. 273) states, "In spite of the absence of our knowledge as to how specific the host-microsporidian relationship might be, the difference in the host animal species must still be used at present as one of the characteristics in the identification of species of Microsporidia."

Hosts

The geographic and host distribution of the Microsporidia has been compiled by Kudo (31). In that paper he shows that Microsporidia have been described from practically all parts

of the world, while N. bombycis and N. apis have been found wherever their hosts exist. Their host distribution includes animals from the lowest to the highest phyla of the animal kingdom. The Microsporidia have been recorded in other Sporozoa and in Ciliata; in the phylum Platyhelminthes from Trematoda and Cestoda; and in the phyla Bryozoa, Trochelminthes, Nemathelminthes, Annelida, Arthropoda, and Chordata. The greatest number of host species has been reported in the phylum Arthropoda from the classes Chilopoda, Crustacea, Arachnoidea, and Insecta. In the class Insecta, the orders Collembola, Thysanura, Isoptera, Orthoptera, Ephemerida, Trichoptera, Lepidoptera, Coleoptera, Hymenoptera, Siphonaptera, and Diptera all have host species. In the phylum Chordata, species in the classes Pisces, Amphibia and Reptilia are attacked by these parasites. Since Kudo's compilation, Poisson (51) reported a microsporidian in the Hemiptera and Fantham, Porter and Richardson (8) in the Odonata.

TAXONOMY OF THE PARASITE

Specific diagnosis: Nosema cynaea Sp. nov., phylum Protozoa, class Sporozoa, subclass Cnidosporidia, order Microsporidia, suborder Monocnidea, family Nosematidae, genus Nosema; in fat body and haemocytes of larval, pupal and adult stages of Cynaeus angustus Lec., family Tenebrionidae, order Coleoptera; rarely observed in the ovary. Spores in salt solution ovoidal with rounded ends, variations to subcylindrical, slightly pyriform, oval, and spherical, circular in optical cross section; average size 4.66 x 3.21 microns, range in length 4.32 to 6.48 microns and in width 2.7 to 4.32 microns; spore membrane highly refractive, of one piece, smooth, structureless, of uniform thickness, composed of chitin; internal details of spore not visible. Fixed spores stained with iron hematoxylin; same form as above, average size 4.02 x 2.73 microns, range in length 3.24 to 5.94 microns and in width 2.16 to 3.78 microns; contents stained blue-black, lighter in anterior part, basal portion of polar filament visible, membrane sharply defined and transparent; immature spores with clear areas in both ends. Stained with Ehrlich's hematoxylin; light bluish in color with two darkly stained areas in one end and a single area in the opposite end. Stained with Feulgen's and fast green FCF; spores generally highly refractive, spore membrane occasionally

stained heavily with fast green; sporoplasm bi-nucleated 1 to 1.5 microns located in posterior portion of spore. Spores in 95 per cent alcohol; clear space at each end, space in anterior portion divided by base of polar filament.

Polar filaments fine, uniform thickness throughout, average length 82.6 microns with range 55.8 to 114.7 microns; polar capsule, if present, lining entire inner surface of spore membrane. Amoebula in salt solution 3.95 x 3.66 microns, in egg albumen and insect haemolymph 1 to 2 microns; spherical in shape and bi-nucleated. Planont stage not recognized. Meront intracellular, 1 to 4 microns, young stages oval with compact eccentric nucleus, older, spherical, nucleus centrally located; sporont typically elliptical, 4 to 6 microns; sporoblast, 4 to 6 microns, 4-nucleated. Each sporont develops into a sporoblast and in turn into one spore.

Locality: Ames, Iowa, U.S.A. March, 1946; collected by Jack L. Krall.

Host: All stages except the egg of C. angustus Lec. Diseased insects display symptoms of milky white opacity; dark spots in the adipose tissue visible through the integument. Distortion of host body wall occurs. Chromatin of host cells assumes a shapeless mass. Infection occurs when the host ingests the spores; and heavy infection results in death of the host.

Type specimens: Slides in author's collection at New York State College of Forestry, Department of Forest Entomology, Syracuse, New York.

EXPERIMENTAL

Materials and Methods

The insects used in this study were reared from adults found in stored corn at the Iowa State College Agronomy Farm near Ames, Iowa, during March, 1946. Stock cultures were established in one-quart, screw-top, fruit jars, and maintained at room temperatures. Each jar contained 1 to 2 inches of water-soaked, whole corn, and on top of this corn a 4 to 5 inch layer of dry cracked corn. The lids for these jars had fine-mesh, copper screen soldered into them to prevent the insects from escaping and to facilitate air movement. As the soaked corn soon sprouted and decayed, moisture and fungus gradients between the two layers were established and these conditions were conducive to the rapid development of the insects. In order to maintain a constant supply of insects, it was necessary to subculture when populations increased; when the corn dried out; or, as frequently occurred, an excessive amount of moisture built up.

Diseased cultures were started by placing two or three larvae which died of the disease into the jars of corn along with living adults. Healthy cultures of insects were established by placing apparently disease-free last instar larvae in separate vials until they transformed to adults. These adults were then placed into jars of corn prepared as

described above, except that the jars were sterilized and the corn had been heat treated at 70°C. for 2 to 4 hours. The jars were stoppered with plugs of cotton to eliminate the possibility of contamination by the spores of the parasite. These cultures were subjected to more or less frequent visual inspections throughout the course of this study. If any jars were found to contain diseased larvae, they were set aside and the insects from these jars were not used in those studies for which healthy individuals were required.

First instar larvae for experimentation were obtained by the simple and excellent method of placing, for oviposition, 30 to 50 adults from healthy and/or diseased cultures into petri dishes which contained corn intermingled with squares of cheesecloth. This procedure was developed because the observations of Krall and Decker (22) showed that the eggs were coated with a viscid material when they were deposited and the beetles preferred to place them in protected places. As the cheesecloth was folded and crumpled, it provided many sheltered locations into which eggs were inserted. The squares of cloth with the adhering eggs were removed after 24 to 48 hours, brushed free of corn, and placed into clean, empty, petri dishes. This method supplied first instar larvae which had no opportunity to obtain food and, as subsequent tests showed, were disease-free.

Experimental studies on the life cycle of the parasite and its effect on the host were conducted at a temperature of 30°C. ± 0.5°C. and a relative humidity of 80 per cent. This humidity was maintained in 6 x 5 inch Scheibler desiccators and in 9 x 8 inch Hopkins-Columbia type museum jars containing a solution of KOH as recommended by Buxton (4). In the desiccators a solution containing 25 gms. of KOH and 100 ml. of distilled water was used in each; and in the museum jars, 50 gms. of KOH and 200 ml. of water. The museum jars were fitted with circular plates of glass supported 2 inches above the solution by bottles. The insects were caged in 45 x 15 mm. homeopathic vials. The desiccators and museum jars were aerated every 2 or 3 days.

The length of the parasite's life cycle was determined by using first instar larvae, larvae in the last three instars of development and adults. The first instar larvae were caged five to a vial and the large larvae and adults were segregated one to a vial. In studying the effects of parasitism, the larvae to be infected and the controls were caged singly.

The food media for the experimental studies were Quaker brand enriched, degerminated, yellow corn meal, and a mixture of this corn meal with finely crushed bodies of larvae and/or adults which had died as a result of infection. Corn meal was used as it was finely ground and the possibility of injury to small larvae would not be a consideration. In addition, the

larvae could be examined without the necessity of removing them from the vials. If cracked corn is used, small larvae may be hidden among the particles thus making examinations difficult. According to the manufacturer's label, 8 ounces of the meal provided the following portions of the human minimum daily vitamin and mineral requirements: Vitamin B_1, 100 per cent; Vitamin B_2, 30 per cent; Iron, 65 per cent; and 8 milligrams of Niacin. (These are included not because the percentages apply to insects but to identify the food used.)

The spore-meal mixture was prepared by mixing thoroughly 1 part of the crushed insects with 24 parts of corn meal. The insects used in the preparation of this food medium were collected from diseased cultures and had been dead for 1 to 3 months. In one experiment, larvae and adults were used which had been dead for 17 to 18 months and stored in a vial at room temperature.

In the parasite life cycle studies, each vial was filled to a depth of approximately 5 mm. with the spore-meal mixture. In the studies on the effects of parasitism, each vial, holding larvae to be infected, contained approximately 1 mm. of the spore-meal medium. An equal amount of corn meal was in the vials containing the control insects. After 5 days, corn meal was added to each vial in the parasitism study groups to bring the total depth of food material to approximately 10 mm.

The stages in the life cycle of the parasite, the seats of infection in the host, and the effect of parasitism on the host tissues were observed and studied in both fixed and living material. Bouin's picro-formol, Carnoy's 1-3-6 mixture and Zenker's fluid were used as fixatives in preparing permanent slides of smears and sections. Heidenhain's iron hematoxylin, Ehrlich's acid hematoxylin, prepared and used according to Guyer (15), and De Tomasi's modification of Feulgen's stain (6) were used as nuclear stains. A 1.0 per cent aqueous solution of orange G or a 0.5 per cent alcoholic solution of fast green FCF were used as counterstains with Feulgen's. A 1.5 per cent solution of crystal violet in 95 per cent alcohol was used to stain polar filaments in temporary mounts. All of these stains, with the exception of the basic fuchsin used in preparing Feulgen's stain, were products certified by the Biological Stain Commission, Geneva, N. Y. The basic fuchsin, lot No. 440701, a product of The Coleman and Bell Company, Norwood, Ohio, was found satisfactory for this technique. The van Wisselingh test for chitin as described by Wigglesworth (59), was used to determine whether the spore membrane and the amber brown substance in which masses of spores became embedded contained chitin.

Serial sections were prepared from insects embedded in paraffin as described by Becker and Roudabush (3) or from specimens double-embedded in celloidin and paraffin according

to Gage (11). The most satisfactory sections of larvae were obtained when the head and the terminal segments of the abdomen were removed after fixation and before embedding in paraffin. This procedure produced a thorough infiltration of the tissues by the paraffin, and, as a result, there was a minimum of tearing and distortion when sections were cut. Sections of the adult abdomen were prepared by using both techniques. If the paraffin embedding technique was used, it was necessary to remove the heavily sclerotized ventral abdominal segments after fixation in order to obtain useful sections. However, when abdomens of adults were double-embedded, excellent sections were obtained without removing the ventral integument.

The extrusion of the polar filament was observed in temporary mounts. These were prepared by crushing a piece of the body of an insect which had died as a result of infection, in a drop of either egg albumen, distilled or tap water, insect haemolymph, or a salt solution. This salt solution contained the following:

$NaHCO_3$	0.06 gms.
KCl	0.06 gms.
$CaCl_2$	0.12 gms.
NaCl	4.2 gms.
Distilled H_2O	300 ml.

Haemolymph was obtained by pricking the integument of a living larva and the blood which oozed through the rupture was applied directly to the spores on a slide. The above media

were also used for the observation of filament extrusion by spores taken from living larvae. A diseased larva was placed in a drop of the medium and the integument was ruptured with forceps. The larva was then removed from the slide and the cover slip applied. To facilitate the preparation of mounts using haemolymph, the same insect supplied both the spores and the blood. The integument of the larva was ruptured by pressure on the cover slip and after a sufficient amount of haemocoelic contents flowed through the rupture, the larva was removed and the cover slip replaced.

Permanent mounts to show polar filaments were prepared by using either distilled or tap water, salt solution, or egg albumen, and spores from dead insects. The slides prepared with egg albumen were placed into a covered petri dish, containing water, for 15 to 20 minutes before they were immersed in a fixative. The water in the petri dish prevented excessive desiccation of the albumen during the time necessary to bring about extrusion by the maximum number of spores. The slides prepared with the other media were air-dried until no movement of moisture was observed when they were held in a vertical position. This ordinarily required 15 to 20 minutes and while the smears were still moist they were immersed in a fixative. When this procedure was followed, the smear adhered to the cover slip after immersion in the fixative, which was not the case when the smear was too moist.

Smears of larval and adult tissues were prepared by crushing the whole insect between slides and quickly immersing them in a fixative. Haemolymph smears, for the study of haemocytes, were prepared exclusively from larvae as it was difficult to obtain a drop of blood from adults. The larva was killed by placing it in a vial which was inserted into a bottle containing a small amount of glacial acetic acid and stoppered. The fumes given off by the acid killed the insect and fixed the haemocytes without coagulation of the plasma. A small drop of salt solution was placed near the edge of a cover slip and the drop of blood, attached to the severed end of a thoracic leg, applied to it. The salt solution diluted the haemolymph and supplied enough moisture to permit the mixture to be spread evenly with a needle before it dried. Before further fixing, these preparations were air-dried until only a film of moisture remained.

The optical equipment used during this investigation was an Ernst Leitz compound binocular microscope with periplane 12 X and 15 X oculars; apochromatic objectives with a focal length of 2 mm. and numerical aperatures of 1.32 and 1.40; and a substage condenser of numerical aperature 1.40. Measurements were made with an ocular micrometer. The polar filaments, however, were measured by stepping off, with a pair of dividers, the tracings made of them with an Abbe camera lucida. The tracings of the polar filaments were made from smears fixed in

Bouin's. The picric acid was not removed with lithium carbonate. These slides were stained with iron hematoxylin and not destained. This method was decidedly more advantageous for the purpose of drawing polar filaments than the standard procedure of removing the picric acid before staining, followed by destaining, as the filaments were darkly stained and consequently easier to trace.

The camera lucida was also used in the preparation of various figures. These were later enlarged with a projector and retraced. In this way, it was possible to show detail more clearly without being limited by the small size of the camera lucida tracings. The photomicrographs and photographs used in this paper were made by the author. Electron micrographs of polar filaments and spores were made by Mr. Ernest F. Fullam, Knolls Research Laboratory, General Electric Company, Schenectady, New York.

Color determinations of adults which had died of the disease and those which died of other causes were made with the aid of Ridgway's (52) "Color standards and color nomenclature."

The results on the life cycle of the parasite and the gross effects of parasitism on the host were obtained by the direct observation of 1431 insects in various stages of development segregated in vials. In addition, many observations were made on hundreds of insects as removed from

cultures. A total of 450 permanent slides was studied for microscopic information on the stages of the parasite, the seats of infection and the effects of the parasite on the tissues attacked. Many more temporary slides were studied to supplement the information from stained preparations, and to furnish data not observed in fixed materials.

The experimental data discussed in the following pages on the gross effects of parasitism were obtained by pooling the various observations made during the course of this study and are not necessarily the results of any one experiment.

Description of Life Stages of the Parasite

Amoebula

In salt solution, the amoebula was a grayish, more or less spherical, mass of protoplasm. The periphery was denser than the internal portion and a refractile granule was usually visible at the point of attachment to the filament. Measurements of 100 amoebulae stained with iron hematoxylin averaged 2.58 x 2.28 microns and ranged from 1.08 to 4.32 microns in diameter. In salt solution, 118 amoebulae averaged 3.95 x 3.66 microns with a range of 2.10 to 6.30 microns. The majority of the amoebulae swelled as they emerged from the polar filament and many of them burst. Consequently, the average dimensions given above were not indicative of the true size of this stage. The amoebulae observed in egg albumen

and in insect haemolymph were 1 to 2 microns in diameter and agreed closely with the size of fixed sporoplasms.

The amoebulae were bi-nucleated. The nuclei were compact and stained deeply with iron hematoxylin and Feulgen's stain. The cytoplasm stained lightly with iron hematoxylin and intensely with fast green. In smears prepared with salt solution and stained with iron hematoxylin, the amoebulae were often irregular in shape (Fig. 4; B), while in smears prepared with egg albumen the spherical shape was retained. Whether the amoebulae were capable of amoeboid movement was not determined.

Planont

This stage was not identified during the course of this study, although uni-nucleated cells of 1 micron in diameter (Fig. 5; D) were observed in the smears of haemolymph from diseased insects.

Meront

This stage of the parasite was intracellular and the youngest forms observed were oval in shape with an eccentric nucleus (Fig. 5; A,B). They were found in the cytoplasm of fat cells and haemocytes and were surrounded by a clear, vacuole-like area. As the meront grew it assumed a spherical shape and the nucleus became centrally located (Fig. 5; B). The meronts observed ranged in size from 1 to 4 microns.

Sporont and sporoblast

The sporont was formed directly from a meront. A uninucleated meront became elliptical in shape or the meront nucleus divided once before the elliptical shape was assumed (Fig. 5; C,E,F). The sporont nuclei (Fig. 5; B) migrated to opposite poles to initiate the sporoblast stage and each nucleus divided into two (Fig. 5; G). The steps in the development of the spore from the 4-nucleated sporoblast were difficult to interpret. The author believes that the protoplasm pulled away from one side of the cell membrane to assume a reniform shape (Fig. 5; H). Evidently a reorganization of the cytoplasm and nuclei occurred at this time. The protoplasm became distributed at the poles and at the center of the sporoblast. These three areas of protoplasm were connected to one another by strands (Fig. 5; I). In the next step of spore development, the spore membrane, polar capsule, if present, and filament were developed and the bi-nucleated sporoplasm was formed in the posterior half of the spore (Fig. 5; J,K).

Spore

The spores, the most conspicuous stage in the life cycle of the parasite, were for the most part quite uniform in dimensions, ovoidal in shape with rounded ends (Fig. 6; F). However, variations in shape to a subcylindrical, slightly pyriform, oval, and spherical form were observed (Fig. 6; B, C,E,G). All of the spores were circular in optical cross

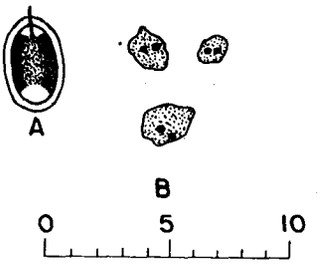

Fig. 4. A; Spore with partially extruded filament; B; Amoebulae; Nosema cynaea. Camera lucida drawing. Scale in microns.

Figure 5

A. Meronts with eccentric nuclei in cytoplasm of fat cell.

B. Meronts and binucleated sporont in cytoplasm of fat cell.

C.F. Bi-nucleated meronts.

D. Uni-nucleated stage found in haemolymph.

E. Meront; showing nuclear division.

G. Sporoblast with 4 nuclei.

H. Sporoblast; cytoplasm contracted from cell membrane.

I. Sporoblast; cytoplasm clumped in three areas.

J. Spore nearly developed.

K. Sporoplasm in spore.

L.M. Stages in germination showing movement of sporoplasm toward anterior end of spore.

N. Haemocyte with eccentric, distorted nucleus and clumped chromatin; three spores in cytoplasm.

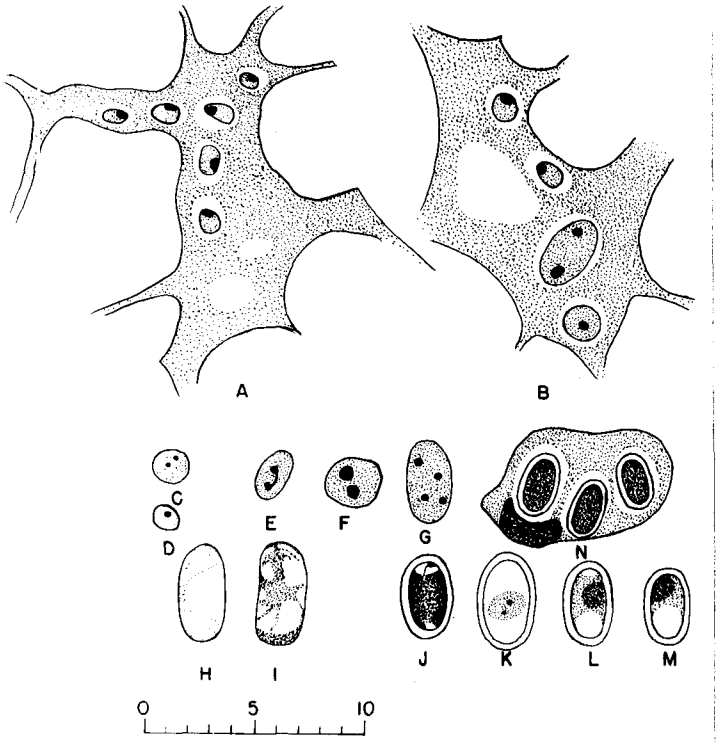

Fig. 5. Stages in the development of Nosema cynaea; showing parasitized fat cells and a haemocyte. Camera lucida drawing. Scale in microns.

section. Measurements of 200 spores in salt solution, 20 from each of 10 dead larvae, averaged 4.66 x 3.21 microns and ranged in length from 4.32 to 6.48 microns and in width from 2.7 to 4.32 microns, while 160 spores, 20 from each of 8 dead larvae, fixed in Bouin's and stained with iron hematoxylin averaged 4.02 x 2.73 microns with a range in length from 3.24 to 5.94 microns and in width from 2.16 to 3.78 microns. The largest spores measured were not common and although their actual measurements were not exceptionally greater than the average, they nevertheless appeared as giants among the other spores (Fig. 7).

The living spores mounted in the media used to cause germination were so highly refractive that it was necessary to reduce the intensity of light in order to see them clearly. The spore membrane was of one piece, smooth, structureless, of uniform thickness, and presumably composed of chitin. Although the thickness of the membrane was visible, its refractivity effectively masked from view any detail of internal structure. No vacuoles were observed at either end of the spore until 5 to 10 minutes after the smears were prepared, when many spores were observed with a clear space in the posterior end. After the filament was extruded the spores lost their turgid appearance and the inner portion appeared sunken.

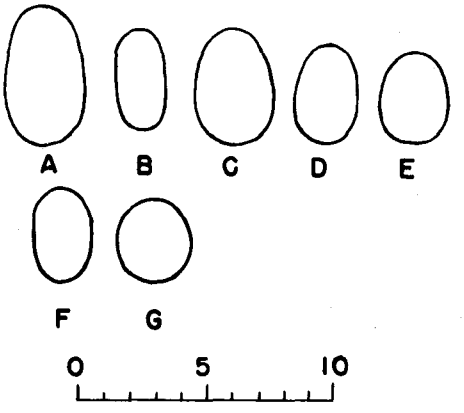

Fig. 6. Variations in size and shape of the spores of Nosema cynaea. Camera lucida drawing. Scale in microns.

Fig. 7. "Giant" spore Nosema cynaea. Heidenhain's iron hematoxylin. 3500 X.

The spores smeared in 95 per cent alcohol were similar in appearance to the spores mounted in the media used for germination, except that the spore membrane was more sharply defined. In a few minutes after preparation, vacuole-like areas were visible at the extremities of the spore. The clear space in the anterior end was bisected by the basal portion of the polar filament (Fig. 8; B). This portion of the filament and the material between the clear areas merged with each other and had the appearance of egg albumen.

Spores stained with iron hematoxylin, after destaining were a uniform blue-black in color with the membrane sharply defined and transparent. A lighter stained area in the anterior end was visible with careful focusing of the microscope. Numerous spores with clear areas in both ends were found in smears and cross sections of diseased insects. These were believed to be immature spores. The spores stained with Ehrlich's acid hematoxylin were light bluish in color with two darkly stained areas in one end and a single area at the opposite end (Fig. 8; A). A similar condition was observed in some of the spores present in larval blood smears stained with iron hematoxylin. These areas were not nuclear.

The spores stained with Feulgen's and fast green presented an entirely different appearance compared to those stained with the hematoxylins. The cytoplasm and polar filament

Figure 8

A. Based on spores stained with Ehrlich's acid hematoxylin.

B. Based on spores in 95 per cent alcohol.

C. Composite of observations on spores stained with Heidenhain's iron hematoxylin and Feulgen's with fast green FCF.

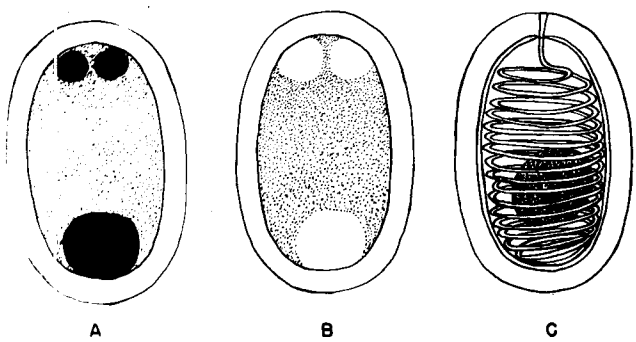

Fig. 8. Spores of Nosema cynaea. 9,000 X.

stained very lightly or not at all in most spores and it was necessary to reduce the light in order to see the shape of the spores. The membrane of some spores stained very darkly with fast green. The nuclear material was conspicuous and consisted of two compact nuclei located in the posterior part of the spore. Careful focusing revealed a clear area around the nuclei of some spores. The spores penetrated by the fast green showed a spherical to oval mass of uniformly stained protoplasm 1 to 1.5 microns in the largest dimension in which the nuclei were embedded. This bi-nucleated mass of protoplasm is the sporoplasm of the spore (Fig. 5; K). In cross section, the sporoplasm was circular and not connected with the inner parts of the spore.

A polar capsule was not observed under any conditions. In the spores treated with 95 per cent alcohol or stained with iron hematoxylin only the basal portion of the polar filament was visible. Germinated spores in hematoxylin-stained preparations were very lightly stained except for a thin, black layer lining the membrane. In some instances, this layer was pulled away from the spore membrane and was suspended, sac-like, in the spore. These observations, and those made of fixed, hematoxylin-stained spores in various stages of germination led the author to believe that the polar capsule in this species, if present, lined the spore membrane and the filament and sporoplasm were located in it. As no one of the

techniques used in this study showed the complete details of a spore figure 8C was drawn to incorporate the various observations made and to show what were believed to be the typical details in the spore of this parasite.

Development of the Parasite and Its Effect on the Host

Spore germination

The spores of Microsporidia, when ingested by a suitable host, germinated in the digestive tract and initiated a new infection. The process of germination involves the extrusion of the polar filament and the escape of the sporoplasm. The attempts of the author to observe germination of the spores of this parasite in the digestive system of adult insects were unsuccessful due to its small size. Consequently, germination of spores was observed in tap water, distilled water, salt solution, egg albumen, and insect haemolymph.

The majority of the spores extruded the polar filament within 15 minutes after a smear was prepared, although in some instances extrusion took place within 2 minutes, while in others not until 45 minutes had elapsed. The appearance of a clear space in the posterior end of a spore, and the development of a blister-like protuberance from the anterior end were the first indications that germination was about to take place. This was plainly evident in iron hematoxylin-stained preparations, whether germination occurred under

Fig. 9. Spores of Nosema cynaea; showing spores A and B in initial stage of germination. Clear area posterior and blister-like protuberance anterior. Heidenhain's iron hematoxylin. 3400 X.

artificial conditions or in the gut of experimentally infected insects (Fig. 9; A,B). This clear area became progressively larger as the filament was extruded until the entire inside of the spore was clear (Fig. 10). Permanent mounts of spore smears stained with Feulgen's and fast green showed that the sporoplasm moved toward the anterior end of the spore as the filament was extruded (Fig. 5; L,M). At the moment of germination, the spores underwent an agitated, whirling movement. It was difficult to observe the entire process of germination of an individual spore because of the motion of the spores and the movement of the polar filaments out of the plane of focus. At the time of most active germination of the spores in water or salt solution, many grayish spheres suddenly appeared in the field. Each sphere was attached to the distal end of a polar filament. This observation reminded the author of the effect produced when a number of balloons were released below a water surface. These spheres were the amoebula stage of the parasite. Occasionally, due to carelessness when a cover-slip was applied to a smear, many air bubbles were formed. These bubbles usually contained many spores surrounded by a thin film of moisture. The spores, in this condition, remained stationary during germination and the filaments were extruded slowly and in one plane of focus. The extrusion of the filament reminded the author of pulling a line from a reel. At the moment the filament was fully extruded, a

substance, differing in appearance from the surrounding medium, spilled from the distal end. This was believed to be the protoplasmic content of an amoebula which had ruptured under these conditions.

The filaments remained attached to the anterior end of the spore in the majority of cases. The fully extruded filaments were extremely fine and of uniform thickness throughout. Measurements of 100 fully extruded filaments, based on the presence of an amoebula at the distal end, averaged 82.6 microns in length, ranging from 55.8 to 114.7 microns. The diameter of a filament was so small that it was not possible to measure it. The filaments showed many windings shortly after extrusion but these later straightened out (Fig. 10 and Fig. 11). The windings have been considered as indicative of the number of turns of the filament inside the spore. Counts made on 20 filaments averaged 20 windings and ranged from 16 to 26. On this basis, the filaments must be closely coiled in the spore and this may be the reason why ungerminated spores stained so darkly with iron hematoxylin and why only the basal portion of the filament was seen.

The unstained, extruded filaments were readily seen at a magnification of 500 X, if the light intensity was reduced. They were refractile or grayish depending on the plane of focus and looked like flagella or cilia under the same conditions. Polar filaments in unfixed and unstained air-dried

Fig. 10. Germinated spore of Nosema cynaea; showing polar filament with amoebula at distal end. Heidenhain's iron hematoxylin. 1400 X.

Fig. 11. Germinated spore of Nosema cynaea; showing windings in the polar filament. Heidenhain's iron hematoxylin. 1600 X.

mounts of spores smeared in distilled water had the same appearance. Filaments stained blue-black with iron hematoxylin and were visible at a magnification of 144 X. Fast green was very unsatisfactory, the filaments staining very lightly or not at all.

It was not possible to determine the internal or external structure of the filaments with a light microscope. Electron micrographs showed that the filaments were smooth externally, and circular in cross section. Three of the filaments showed a lighter area running through the center of them and appeared tubular. Mr. Fullam (10), however, pointed out that these could well be artifacts and many more observations would be necessary before the tubular character of the filaments was established by the use of the electron microscope. Nevertheless, it is believed that the polar filaments are tubular because filaments were seen shooting across the field of the microscope and at the moment of completed extrusion the amoebulae emerged from the ends of the filaments. It is also believed that the filaments are evaginated during extrusion because the fully extruded, stained filaments were smaller in diameter than the partially extruded ones. Also, if the filaments were not evaginated, then the proximal end of the extruded filament would of necessity possess some structure to keep it attached to the spore. No such structure was present.

Length of life cycle

An approximation of the time required for this organism to complete its development was obtained by experimentally infecting 295 larvae in the first instar, 10 larvae in the last three instars of development and 16 adults. The adults were obtained by rearing first instar larvae on corn meal. The developmental time was first determined with first instar larvae. The large larvae and adults were then used to determine what differences existed in the developmental time of the parasite in these stages as compared to the first instar larvae.

A number of first instar larvae were removed daily from the spore-meal mixture, brushed free of corn meal or particles of dead insects, smeared and examined microscopically. The smears were prepared by placing a larva into a drop of salt solution and rupturing the integument by applying pressure to the cover-slip. In order to eliminate errors due to the presence of spores adhering to the integument, the life cycle was not considered to be completed unless spores were found either in the tissues or oozing through the ruptured integument.

The shortest developmental time for the parasite in first instar larvae was found to be 8 days in each of 23 larvae. Consequently, the large larvae and adults were permitted to feed 8 days before smears were made. In these stages, forceps were used to crush the insects when smears were prepared. In

the large larvae, the shortest developmental time observed was 11 days in one larva; while, in the adult stage, the parasite completed its development in 10 days in each of three adults.

Some of the small larvae were permitted to feed up to 12 days; large larvae up to 17 days; and adults for 11 days. On a comparative basis, there was, for the most part, a decided daily increase in the number of spores present in the small larvae. This was not true with the large larvae and adults, as fewer spores were present in some individuals which fed for a longer time than others.

The larvae and adults of Cynaeus angustus become infected when the spores of the parasite are ingested and germinate in the digestive tract. Presumably, the amoebulae reach the haemocoel by passing through the midgut or the Malpighian tubules. This activity was not directly observed. The lobes of the fat body in close proximity to the midgut or a Malpighian tubule were the first tissue to be invaded by the parasite. Each amoebula, after it entered the cytoplasm of the fat cells, became a meront that grew and multiplied by binary fission. These meronts eventually transformed into elliptical sporonts with a nucleus at each pole. These sporonts transformed into sporoblasts by the division of each nucleus into two. Further development was difficult to follow, but the author believed that the cytoplasm and the nuclei underwent a reorganization

during the formation of the spore membrane, polar capsule, filament, and the sporoplasm.

Mode of infection and dissemination of spores

The author observed that first instar larvae of C. angustus were readily infected when they fed on a spore-meal mixture. In the transmission experiments, 237 larvae which lived for at least 10 days after exposure to infection were found to be diseased. One exception to this was a larva which was removed from the spore-meal mixture after 24 hours exposure to infection. This larva continued its development on corn meal and was found to be healthy when a smear was examined microscopically.

Although spores were found in the ovary of a diseased adult female, it is believed that germinative infection seldom, if ever, occurs. This belief is substantiated by the fact that only one of the many larvae used as controls became diseased. If germinative infection was a consideration, it was presumed that more controls would have been diseased since first instar larvae hatched from eggs deposited by both healthy and diseased adults were used as controls.

Under experimental conditions, insects were forced to feed on the spores of the parasite. To determine whether or not the host voluntarily fed on diseased insects, 213 dead larvae and 97 dead adults were removed at random from 4

diseased cultures of insects. A thorough examination of these individuals showed conclusive evidence of feeding on 201 larvae and none on 12 larvae or on the adults. Undoubtedly some of the adults had been fed upon, but the loss of appendages was not accepted as evidence of such. Microscopic examination of smears prepared from these insects showed many spores present in all of the larvae and in 80 adults.

As a result of this feeding activity, the living insects became infected and were responsible for disseminating the spores through the food medium. Microscopic examination of larvae taken from a spore-meal mixture showed the spores adhering to the appendages, mouthparts and integument. As these surface contaminated insects wandered through the food medium, spores were brushed off, and in the case of moulting larvae, contaminated exuviae were also left as a source of infection. Finally, the physical disintegration of the dead, diseased insects also released spores to contaminate the food medium.

It was believed that the feces also played a part in dissemination, as spores with unextruded polar filaments were numerous in cross sections of the hind-gut. Although the viability of these spores was not proved, they stained the same with iron hematoxylin as those from living or dead insects.

A predacious mite, identified by Baker (1) as Blattisocius triodons Keegan, family Laelaptidae, was observed in some of the insect cultures. Although this mite was not observed feeding on living insects, many of them were found on recently dead diseased insects that were still soft and moist. No attempt was made to incriminate this mite with the direct transmission of infection from host to host, but spores were found on the integument of many individual mites. In any event, the mite served to spread the spores through the food medium.

Macroscopic symptoms of disease

The presence of circular to oval or irregular black spots visible through the integument of larvae was determined to be a reliable symptom of infection (Fig. 12). As a result of this observation, larvae, pupae and adults from diseased cultures were examined for these and any other abnormalities that could be used as diagnostic characters of the disease. Any of the host stages found to be abnormal in appearance were smeared and examined microscopically for the presence or the absence of spores. The presence of blackened areas in pupae or adults was as reliable a symptom of disease in these stages as in the larvae.

In the adult, the heavily pigmented integument and the elytra masked the black spots. However, if present, the spots

were often visible through the ventral abdominal segments and readily seen through the transparent dorsal integument of the abdomen when the elytra were raised or removed. As subsequent experiments showed, larvae which were infected for a considerable period of time seldom transformed to pupae and consequently fewer observations of this stage were possible.

An opaque milky appearance was found to be an indication of infection in both larvae and adults, but was not necessarily indicative of a diseased condition in pupae as the normal coloration of this stage is white. This coloration was only visible in the adult when the elytra were raised to expose the dorsal portion of the abdomen. This opacity was due to the presence of many spores in the normally translucent fat body.

The body was distorted in many diseased individuals. Some of the larvae were fusiform in shape because the mid-section was swollen; while, in others, lateral distention of various segments was observed (Fig. 12). Distortion of diseased pupae consisted of an elongation of the abdomen. In adults, the innumerable spores caused the abdomen to swell upward and to elongate, with the result that the elytra were raised and the tip of the abdomen was exposed.

Although individual insects with all three symptoms were observed, opacity was usually the first symptom to appear, followed by black spots with or without distortion of the integument.

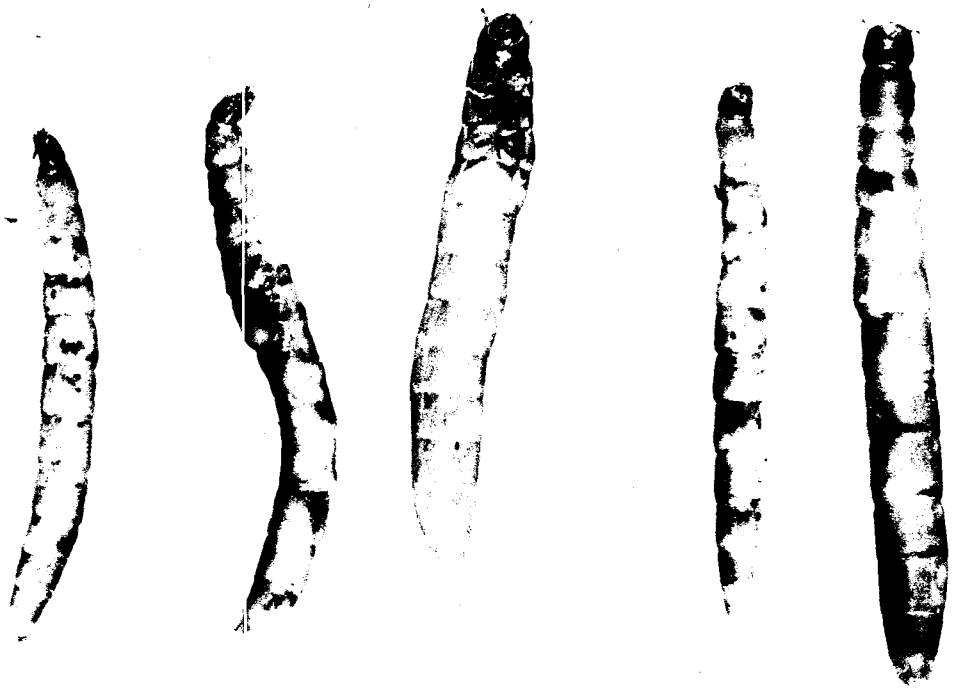

Fig. 12. Diseased larvae of Cynaeus angustus Lec.; showing black spots and distortion of integument. 8 X.

Reliability of macroscopic symptoms in diagnosis

Although insects with abnormal coloration or distortion were all found to be diseased on microscopic examination, tests were conducted to check further the reliability of these abnormalities as symptoms of infection, and to see if the larvae would transform to adults. A total of 105 larvae with black spots and 25 larvae from a healthy culture were segregated one to a vial on corn meal. Of the black-spotted individuals, 104 died in the larval stage, and one died after transforming to the pupal stage. The 25 larvae from a healthy culture all transformed to the adult stage. Spores were found in the smears of the insects with black spots and none in those from the healthy culture.

Time required for spots to appear in larvae

The most readily observed disease symptom in larvae was the presence of black spots. To determine when this symptom appeared after larvae were exposed to infection and how soon thereafter they died, observations were made on larvae infected during the first and last three instars of development. Some of the larvae, infected in the first instar, fed on a spore-meal mixture until death and others were transferred, after 24 hours, to corn meal. The large larvae, however, all fed on the spore-meal mixture until they died.

The larvae were examined weekly with a dissecting microscope at a magnification of 12 X. The time required for the

spots to appear is recorded in Table 1, and the number of days after the appearance of spots until death occurred are given in Table 2.

Table 1. Time lapse after exposure to infection for black spots to appear in larvae of Cynaeus angustus

Days ± 3 after exposure to infection	Number of larvae showing spots I	II	III	Days ± 3 after exposure to infection	Number of larvae showing spots I	II	III
11	-	1	-	32	-	11	-
15	1	-	-	34	-	-	1
16	-	-	3	36	1	-	-
18	-	1	-	37	-	-	1
20	3	-	3	39	-	7	-
22	2	-	-	41	1	-	-
23	-	-	10	46	-	4	-
25	-	14	-	51	-	-	1
27	2	-	4	74	-	1	-
30	-	-	7				
Total					10	39	30
Average Days					25.0	32.3	25.9

I Larvae in first instar and fed spore-meal until death.
II Larvae in first instar and fed spore-meal for 24 hours then fed corn meal until death.
III Larvae in last three instars and fed spore-meal until death.

The black spots were small when they first appeared and were circular to oval in shape. These spots became progressively larger until many of them coalesced to form large

black areas in the larvae.

Table 2. Length of larval life of Cynaeus angustus after appearance of black spots

Days ± 3 after appearance of spots	Number of larvae showing spots I	II	III	Days ± 3 after appearance of spots	Number of larvae showing spots I	II	III
4	6	7	5	39	-	2	-
10	3	-	1	40	-	1	-
11	-	6	8	46	1	-	-
18	-	6	10	53	-	4	-
21	-	1	-	60	-	1	-
25	-	2	4	67	-	2	-
32	-	6	1	81	-	1	-
Total					10	39	29
Average Days					10.3	27.4	14.8

I Larvae in first instar and fed spore-meal until death.
II Larvae in first instar and fed spore-meal for 24 hours then fed corn meal until death.
III Larvae in last three instars and fed spore-meal until death.

The infected larvae were not hampered in their movements until shortly before death when they became sluggish and un-coordinated in their activities. At this stage, larvae were unable to right themselves even though the thoracic legs were capable of quite vigorous movements. This reduction of activity was evidently because of a great reduction in

the function of the body muscles due to pressure exerted by the countless numbers of spores in the fat body.

Effect of parasitism on larval weight

It was noticed that larvae on a spore-meal mixture generally appeared smaller in size than the controls of the same age. To find out whether there was any significant weight difference between healthy and diseased larvae of the same age two experiments were carried out.

In one case, 90 first instar larvae were segregated 1 to a vial. To 50 of these vials a spore-meal mixture, prepared from adults which had been dead for approximately 17 to 18 months, was added to a depth of 1 mm., and the same volume of corn meal to the other 40 vials. After 5 days, corn meal was added to each of the 90 vials to bring the total amount of food to a depth of 5 to 7 mm. Of the 90 larvae, 16 died before the end of the 30 day treatment period. The remaining 74 were weighed, and examined for the presence of spores. The larvae on the spore-meal mixture were all found to be diseased while those on the corn meal were all healthy. The weights and the number of larvae in each weight class are recorded in Table 3.

Table 3. Comparative weights of larvae of Cynaeus angustus fed on spore-meal mixture or on corn meal for 30 days

Weight in milligrams	Number of larvae Diseased	Healthy	Weight in milligrams	Number of larvae Diseased	Healthy	Weight in milligrams	Number of larvae Diseased	Healthy
0.3	2	-	2.3	1	-	6.4	-	2
0.5	2	1	2.4	2	-	6.8	-	3
0.6	1	-	2.8	1	-	6.9	-	1
0.7	2	-	2.9	1	-	7.1	-	1
0.8	1	-	3.5	-	1	7.4	-	2
1.0	1	-	3.6	-	1	7.7	-	2
1.2	3	-	4.6	-	1	7.8	-	2
1.3	7	-	4.7	-	1	8.1	-	1
1.4	5	-	5.1	-	1	8.3	-	1
1.7	2	-	5.3	-	1	8.5	-	1
1.8	3	-	5.4	-	1	8.6	-	1
2.0	1	-	5.8	-	1	8.7	-	1
2.1	5	-	6.2	-	2	8.9	-	1
2.2	1	-	6.3	-	1	9.6	-	2

Total: Diseased, 41; Healthy, 33
Average Weight: Diseased, 1.5 mg.; Healthy, 6.7 mg.

In the other experiment, 68 first instar larvae were segregated in vials containing a spore-meal mixture prepared as above. These larvae were permitted to feed for 24 hours and at the end of that time were transferred to vials containing 1 millimeter of corn meal. On the fifth day the amount of corn meal was increased to a depth of 5 to 7 mm. At the end of 30 days the living larvae were weighed and examined for spores. A total of 34 larvae were found to be

diseased and 22 were healthy. The results are summarized in Table 4.

Table 4. Comparative weights of larvae of Cynaeus angustus fed 24 hours on spore-meal and 29 days on corn meal

Weight in milligrams	Number of larvae Diseased	Healthy	Weight in milligrams	Number of larvae Diseased	Healthy	Weight in milligrams	Number of larvae Diseased	Healthy
0.8	1	-	3.2	3	1	6.3	1	1
1.2	1	-	3.4	1	-	6.4	2	1
1.3	1	-	3.5	1	-	6.5	-	1
1.4	1	-	3.6	1	-	7.6	-	2
1.7	1	-	4.1	-	2	7.7	1	-
2.2	2	-	4.2	2	1	7.8	-	2
2.4	1	1	4.3	3	-	7.9	-	1
2.5	2	-	4.4	-	1	8.0	-	1
2.6	1	-	4.7	-	1	8.2	-	1
2.7	-	1	5.0	1	-	8.5	1	-
2.8	-	1	5.2	3	-	9.3	-	1
2.9	1	-	5.4	-	1			
3.1	1	-	6.2	1	1			

Total: Diseased, 34; Healthy, 22
Average Weight: Diseased, 3.8 mg.; Healthy, 5.8 mg.

A statistical analysis using the "t" test showed a very highly significant difference in the weights of the diseased and healthy larvae recorded in Tables 3 and 4. This test also showed that the difference in weights of the diseased larvae in Table 3 was highly significant when compared to

the weights of the diseased individuals in Table 4. However, a test of the comparative weights of the healthy larvae in the two studies showed no significant difference.

The results recorded in Table 4 also show that many spores lose their viability with age. The author believes this must be the case, because first instar larvae fed on a spore-meal mixture prepared from diseased insects dead for 1 to 3 months all became infected. In this experiment, spores 17 to 18 months old were used and many of the larvae exposed to infection for 24 hours did not become diseased.

Infection early and late in larval life

It was known that when first instar larvae were infected with spores 1 to 3 months old they died before transforming to the pupal stage. This was true whether the larvae fed on a spore-meal mixture throughout life or were transferred to corn meal after an initial feeding period of 24 hours on the infective medium. Nevertheless, some diseased pupae and adults were observed in diseased cultures. By observation, it was impossible to determine whether the disease was carried into the adult stage from the larval stage. To determine whether larvae infected late in life carried the infection into the adult stage, 110 healthy larvae in the last three instars of development were segregated in vials on a spore-meal mixture. In the course of this study,

39 larvae transformed to adults and of these, 36 adults were diseased while 3 adults were healthy.

This seems to indicate that infection late in larval life will permit normal development and may be an important source of diseased adults.

Life study of diseased and healthy larvae

The length of time required for an uninfected larva to transform to the adult stage was recorded for all the controls. These data and those on the life duration of larvae infected in the first instar and found to be diseased on death are presented in Table 5.

Table 5. Life duration of diseased larvae of Cynaeus angustus and time for healthy larvae to become adults

Days ± 3 after start of experiment	Larval treatment I	II	III	Days ± 3 after start of experiment	Larval treatment I	II	III
8	4	-	-	56	-	-	4
11	-	2	-	58	-	-	10
13	2	-	-	60	1	6	1
15	3	-	-	61	-	-	3
18	-	2	-	62	-	-	1
19	3	-	-	63	-	-	3
20	5	-	-	65	-	-	2
22	6	-	-	67	4	5	-
25	-	10	-	68	-	-	1
26	7	-	-	69	-	-	1
27	4	-	-	70	-	-	6
29	5	-	-	71	1	-	2
32	-	10	-	74	1	2	1
33	6	-	-	76	-	-	2
34	5	-	-	77	-	-	1
36	2	-	-	78	-	-	1
39	3	17	8	79	-	-	1
41	1	-	-	81	-	2	-
44	-	-	6	82	-	-	1
46	1	5	12	83	-	-	2
47	-	-	2	88	-	1	-
49	-	-	1	90	-	-	1
50	1	-	-	95	-	3	-
51	-	-	19	97	-	-	1
53	2	4	7	102	-	2	-
54	-	-	2	116	-	1	-
55	1	-	1				
Total Insects					66	72	103
Average Days					31.8	47.6	56.3

I Larvae in first instar fed on spore-meal until death.
II Larvae in first instar fed on spore-meal for 24 hours then transferred to corn meal.
III Larvae in first instar fed on corn meal until adults.

The average time of 56.3± 3 days, recorded in Table 5, required for healthy larvae to reach the adult stage coincided quite closely with the average developmental time of 53.6 days for 22 larvae reared on corn meal as reported by Krall and Decker (22).

Microscopic observations of tissues

The parasites attacked the fat body and haemocytes of larvae, pupae and adults. Spores were also found in the germaria of the ovarioles of adult females. The intracellular stages of the parasite were found only in the cytoplasm of the host cells and were surrounded by a clear, vacuole-like area (Fig. 8; A,B).

The fat cells were the principle sites of attack. The initial invasion of the fat body was confined to various lobes of the organ, and, in cross sections of many diseased larvae, the parasite occupied only portions of a lobe. The peripheral lobes of the fat body were usually the last part of this organ to become infected. Evidently more of the organ was invaded as a result of reinfection and auto-infection. As the parasite increased and developed, the fat body became filled with spores (Fig. 13).

Haemocytes containing spores were readily found but it was more difficult to find meronts. The presence of spores in some of the haemocytes may have been a result of phagocytosis

by these cells. However, the author was inclined to believe that these cells had been attacked, since the nuclei were eccentric, distorted and the chromatin material reduced to a shapeless mass (Fig. 5; N). Nuclear hypertrophy or hyperplasia was not observed, but an increase in volume and distortion of the infected cells always resulted as the parasite population increased.

The black spots used for macroscopic diagnosis were found to be masses of spores embedded in an amber brown substance that had the appearance of chitin. These spore masses were surrounded by what appeared to be a "connective tissue" and in some instances by a layer of haemocytes. These masses of spores were most common in the inner portions of the lobes of the fat body although they were also found at the periphery.

A test for the presence of chitin in the amber brown material was made by the van Wisselingh (59) method but with negative results. This substantiated the results obtained by Goodrich (13).

Parasitism and host populations

Although experiments were not conducted for the purpose of determining the effects of parasitism on host populations, some general observations on healthy and diseased cultures of insects were made. Many cultures were started at the same

time and with equal numbers of adults. The sex ratios of these adults were not known. The populations of both healthy and diseased cultures increased to a high level within 40 days and the effects of parasitism in the diseased cultures were not evident until approximately 3 to 4 months after the cultures were started. At that time the insects were sifted from the food material and the number of living insects from the diseased cultures was noticeably less than that from healthy cultures. It was also noted that the diseased cultures contained few or no larvae in the first three instars of development and in the healthy cultures larvae in all stages of development were numerous.

Miscellaneous observations

Larvae and adults which died from the disease were readily recognized. The larvae remained flaccid for 2 to 3 days after death and then became hard and brittle. Instead of becoming shriveled, they retained quite closely their normal size and shape. This was due to the presence of many spores which prevented the collapse of the integument. This was also true of the adults, but was not as outstanding because of the more rigid integument. If the elytra were removed, however, it was seen that the abdomen was distended rather than collapsed as would be the case with adults which died from other causes. In both larvae and adults the body

Fig. 13. Portion of abdomen of diseased adult Cynaeus angustus Lec.; showing spores of Nosema cynaea in fat body. Heidenhain's iron hematoxylin. 300 X.

cavity was filled with a chalky mass of spores.

An additional method of identifying adults dead of the disease was based on the color of the ventral surfaces of the beetle. In the adults which died of the disease, the ventral parts of the body were brazil red, (Ridgway 52), in contrast to the normal chestnut brown coloration of the insects dead of other causes.

Some spores, 25 months old, germinated when mounted in salt solution. There was, however, a noticeable reduction in the number of these spores germinating as compared to smears prepared with spores 1 to 3 months old.

The integument of large diseased larvae ruptured much more readily when pressure was applied to the cover slip than did the integument of healthy larvae of the same age.

SUMMARY AND CONCLUSIONS

1. The microsporidian considered in this paper was placed in the family Nosematidae, genus Nosema and the name Nosema cynaea Sp. nov. proposed for this species.
2. The amoebula, meront, sporont, sporoblast and spore stages in the life cycle of the parasite were identified. The intracellular stages were found in the cytoplasm of the fat cells and haemocytes of the host. The nuclei of infected cells became eccentric and distorted and the chromatin material was reduced to a shapeless mass.
3. The spores germinated under artificial conditions in distilled or tap water, salt solution, or egg albumen. Germination under natural conditions occurred in the haemolymph and digestive tract of the host.
4. The sporoplasm was ejected through the tubular polar filament which was evaginated during germination.
5. Initial infection of the host, Cynaeus angustus, occurred when spores were ingested. Auto-infection occurred when host cells ruptured and the released spores germinated in the haemolymph.
6. The minimum developmental time of the parasite was 8 days in experimentally infected first instar larvae.
7. The macroscopic symptoms of disease were milky white opacity, and adipose tissue with black spots visible through the integument of the host. Distortion of the

integument also occurred. These symptoms were reliable criteria for the diagnosis of the disease in larvae or adults. Opacity, however, was not reliable for the diagnosis in pupae.

8. The black spots, visible through the body wall, were found localized in the fat body and consisted of masses of spores embedded in an amber brown matrix. This matrix was not composed of chitin.
9. The average time required for black spots to appear in experimentally infected larvae was 28.9 $\pm$ 3 days.
10. There was a highly significant difference in the weights of diseased larvae and the weights of healthy larvae of the same age.
11. First instar larvae infected by spores 1 to 3 months old died before transforming to the pupal stage whether they had been exposed to infection for only 24 hours or throughout life. On the other hand, many first instar larvae exposed 24 hours to infection by spores 17 to 18 months old did not become diseased.
12. Larvae in the last three instars of development exposed to infection by spores 1 to 3 months old were able to transform to the adult stage in many cases.
13. Although germination of some spores 25 months old occurred in salt solution, many more spores 1 to 3 months old germinated under the same conditions.

14. Some diseased larvae lived as long as or longer than the average time of 56.3 ± 3 days required for the healthy larvae to transform to the adult stage.

15. Nosema cynaea may be considered an important factor in the biological control of Cynaeus angustus.

16. It was readily ascertained whether larvae and adults died of the disease as the integument inclosed a chalky mass of spores. The larvae were quite normal in shape after death. The ventral surface of the adult was brazil red in contrast to the chestnut brown of the adult which died from other causes.

GLOSSARY

The following terms used to designate the various stages in the life cycle of the Microsporidia were taken in part from Kudo (31, p. 59-60).

Amoebula stage.	The sporoplasm which by amoeboid movements has left the spore membrane, a stage leading up to the schizont.
Anterior end.	The end of the spore from which the polar filament becomes extruded through the foramen. If the two extremities are dissimilar in form, the anterior end is usually more or less attenuated. . . . .
Meront.	Coined by Stempell to designate a schizont of a microsporidian. . . . .
Pansporoblast.	Coined by Gurley (14) to designate in a myxosporidian trophozoite an enclosed area in which two sporoblasts become differentiated. Strictly speaking, therefore, the genera Glugea and Perezia have pansporoblasts in this sense. The term however has also been used to designate in general a grown sporont of the polysporous genera in which two to many sporoblasts are formed.
Planont.	The stage between free amoebula and schizont stages which are found in the alimentary canal or body cavity of the host soon after the spores germinate; coined by Stempell (53).
Polar capsule.	A sac in which the polar filament is coiled. One of the typical structures of a cnidosporidian spore.
Polar filament.	A fine and long filament coiled in the polar capsule, which under suitable stimulation is extruded. . . . .
Posterior end.	The end of a spore opposite the anterior. If the two extremities are dissimilar in form, this is more or less rounded.
Schizogony.	The changes which a schizont undergoes during its asexual reproduction.
Schizont.	Early intracellular stages which multiply by asexual reproduction.

Spore-membrane.	The envelope of a spore composed of a single piece or in some cases of two valves. Sporocyst.
Sporoblast.	A cell which develops directly into a spore.
Sporogony.	The changes in the development of spores from the sporont stage.
Sporont.	An individual which gives rise to one to many sporoblasts.
Sporoplasm.	The sporozoite of a cnidosporidian spore, a protoplasmic mass found inside of the spore. . . . .

LITERATURE CITED

1. Baker, Edward W. — Division of Insect Identification, Washington, D. C. Information on a mite, Blattisocius triodons Keegan. [Private Communication.] 1949.

2. Balbiani, M. — Sur les microsporidies ou psorospermies des articulés. C. R. Acad. Sci. 95: 1168-1171. 1882.

3. Becker, E. R. and R. L. Roudabush. — Brief directions in histological technique. Ames, Iowa, Collegiate Press, Inc. 1939.

4. Buxton, P. A. — The measurement and control of atmospheric humidity in relation to entomological problems. Bull. Ent. Res. 22: 431-447. 1931.

5. Decker, G. C. — U. S. Bur. of Ent. and Plant Quar. Insect pest survey bulletin. 21: 220. 1941.

6. De Tomasi, J. A. — Improving the technic of the Feulgen stain. Stain Tech. 11: 137-144. 1936.

7. Fantham, H. B. and A. Porter. — The morphology and life history of Nosema apis and the significance of its various stages in the so-called "Isle of Wight" disease in bees(Microsporidiosis). Ann. Trop. Med. Paras. 6: 163-195. 1912.

8. _____, A. Porter and L. R. Richardson. — Some Microsporidia found in certain fishes and insects in eastern Canada. Paras. 33: 186-208. 1941.

9. Flu, P. C. — Studien über die im Darm der Stubenfliege, Musca domestica, vorkommenden protozoären Gebilde. Centralbl. Bakt. (I) Orig. 57: 522-535. 1911.

10. Fullam, E. F. Knolls Research Laboratory, General Electric Company, Schenectady, N. Y. Information on the structure of polar filaments. [Private Communication.] 1949.

11. Gage, S. H. The microscope. Dark-field 14th Rev. ed. Ithaca, N. Y., The Comstock Publishing Co. 1925.

12. Goodrich, H. L. M. P. The spore of Thelohania. Arch. Zool. Exp. Gen. 59: 17-19. 1920.

13. _____. Reactions of Gammarus to injury and disease, with notes on some microsporidial and fungoid diseases. Quart. Jour. Micro. Sci. N. S. 72: 325-353. 1929.

14. Gurley, R. R. On the classification of the Myxosporidia, a group of protozoan parasites infesting fishes. Bull. U. S. Fish Comm. 11: 407-420. 1893.

15. Guyer, M. F. Animal micrology. 4th Rev. ed. Chicago, Ill., The University of Chicago Press. 1936.

16. Hatch, M. H. U. S. Bur. of Ent. and Plant Quar. Insect pest survey bulletin. 19: 481. 1939.

17. _____. Stored grain beetles in Western Washington with special reference to the tenebrionid, Cynaeus angustus Lec. Pan-Pacific Ent. 16: 35. 1940.

18. Hertig, M. The normal and pathological histology of the ventriculus of the honeybee, with special reference to infection with Nosema apis. Jour. Paras. 9: 109-140. 1923.

19. Hesse, E. — Sur Myxocystis mràzekia Hesse, microsporidie de Limnodrilus hoffmeisteri Clap. C. R. Soc. Biol. 57: 12-13. 1905

20. Ishiwata, S. — Note on a species of Nosema infecting Attacus cynthia Drury. Jour. Paras. 3: 136-137. 1917.

21. Korke, V. T. — On a Nosema(Nosema pulicis n.s.) parasitic in the dog flea (Ctenocephalus felis). Indian Jour. Med. Res. 3: 725-730. 1916.

22. Krall, J. L. and G. C. Decker. — The biology of Cynaeus angustus Lec., a new stored grain pest. Iowa State Coll. Jour. Sci. 20: 385-402. 1946.

23. Kudo, R. — Contributions to the study of parasitic Protozoa. I. On the structure and life history of Nosema bombycis Nägeli. Bull. Imper. Seric. Exp. Stat. Tokio. 1: 31-51. 1916.

24. _____. — Notes on Nosema apis Zander. Jour. Paras. 7: 85-90. 1920.

25. _____. — Microsporidia parasitic in copepods. Jour. Paras. 7: 137-143. 1921.

26. _____. — On the nature of structures characteristic of cnidosporidian spores. Trans. Amer. Micro. Soc. 40: 59-74. 1921.

27. _____. — Studies on Microsporidia, with special reference to those parasitic in mosquitoes. Jour. Morph. 35: 153-193. 1921.

28. _____. — Studies on Microsporidia parasitic in mosquitoes. II. On the effect of the parasites upon the host body. Jour. Paras. 8: 70-77. 1921.

29. _____. — Microsporidian parasites of ephemerid nymphs. Jour. Paras. 10: 22-24. 1923.

30. _____. Studies on Microsporidia parasitic in mosquitoes. III. On Thelohania legeri Hesse (Th. illinoisensis Kudo). Arch. Protist. 49: 147-162. 1924.

31. _____. A biologic and taxonomic study of the Microsporidia. Illinois Biol. Monogr. 9 (no. 2 and 3): 1-268. 1924.

32. _____. Studies on Microsporidia parasitic in mosquitoes VI. On the development of Thelohania opacita, a culicine parasite. Jour. Paras. 11: 84-88. 1924.

33. _____. Studies on Microsporidia parasitic in mosquitoes. IV. Observations upon the Microsporidia found in the mosquitoes of Georgia, U.S.A. Centralbl. Bakt. Orig. 96: 428-440. 1925.

34. _____. Studies on Microsporidia parasitic in mosquitoes. V. Further observations upon Stempellia (Thelohania) magna Kudo, parasitic in Culex pipiens and C. territans. Biol. Bull. 48: 112-127. 1925.

35. _____. Nosema termitis n. sp., parasitic in Reticulitermes flavipes. Jour. Morph. 73: 265-279. 1943.

36. _____ and D. C. Hetherington. Notes on a microsporidian parasite of a nematode. Jour. Paras. 8: 129-132. 1922.

37. Le Conte, J. L. Descriptions of new species of Coleoptera from California. Ann. Lyceum Nat. History N. Y. 5: 149. 1852.

38. _____. Classification of the Coleoptera of North America, part I. Smithsonian Misc. Coll. 3 (no.136): 233. 1862.

39. Léger, L. and O. Duboscq. Protistes parasites de l'intestin d'une larve de Ptychoptera et leur action sur l'hôte. Bull. Class. Sci. Acad. Royal Belg. 8: 885-902. 1909.

40. Léger, L. and E. Hesse. Cnidosporidies des larves d'éphémères. C. R. Acad. Sci. 150: 411-414. 1910.

41. _____, and _____. Mrazekia, genre nouveau de microsporidies à spores tubuleuses. C. R. Soc. Biol. 79: 345-348. 1916.

42. _____, and _____. Sur la structure de la spore des microsporidies. C. R. Soc. Biol. 79: 1049-1053. 1916.

43. _____, and _____. Microsporidies à spores sphériques. C. R. Acad. Sci. 173: 1419-1421. 1921.

44. _____, and _____. Microsporidies bactériformis et essai de systématique du groupe. C. R. Acad. Sci. 174: 327-330. 1922.

45. Mercier, L. Sur le développment et la structure des spores de Thelohania giardi Henneguy. C. R. Acad. Sci. 146: 34-38. 1908.

46. Nägeli. Nosema bombycis Nägeli. Bot. Zeit. 15: 760. 1857.

47. Nicholson, A. J. The development of the ovary and ovarian egg of a mosquito, Anopheles maculipennis Meig. Quart. Jour. Micro. Sci. N.S. 65: 396-448. 1921.

48. Pasteur, L. Etudes sur la maladie des vers à soie. t. 1. Paris. 1870.

49. Payne, N. M. A parasitic hymenopteron as a vector of an insect disease. Ent. News. 44: 22. 1933.

50. Pérez, C. — Microsporidies parasites des crabes d'Arcachon (Note préliminaire). Soc. Sci. Arcachon, trav. lab. 8: 15-36. 1905.

51. Poisson, R. — Recherches sur les microsporidies parasites des Hémiptères. Arch. Zool. Exp. Gen. 69: 55-63. 1929.

52. Ridgway, R. — Color standards and color nomenclature. Washington, D. C., Pub. by author. 1912.

53. Stempell, W. — Ueber Nosema bombycis Nägeli. Arch. Protist. 16: 281-358. 1909.

54. Strickland, E. H. — Further observations on the parasites of Simulium larvae. Jour. Morph. 24: 43-106. 1913.

55. Thélohan, P. — Observations sur les Myxosporidies et essai de classification de ce organismes. Bull. Soc. Philom. 4: 165-178. 1892.

56. _____. — Recherches sur les Myxosporidies. Bull. Sci. France et Belg. 26: 100-394. 1895.

57. Trager, W. — The hatching of spores of Nosema bombycis Nägeli and the partial development of the organism in tissue cultures. Jour. Paras. 23: 226-227. 1937.

58. Weissenberg, R. — Beiträge zur Kenntnis des Zeugungskreises der Mikrosporidien Glugea anomala Moniez und hertwigi Weissenberg. Arch. Mikr. Anat. 82: Abt. 2: 81-163. 1913.

59. Wigglesworth, V. B. — The principles of insect physiology. 3rd ed. London, Methuen & Co. ltd. 1947.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. W. H. Weilhouse, under whose direction this problem was conducted, for his courtesies and understanding, and to the other members of the committee for their considerate treatment; his appreciation to Mr. E. F. Fullam, Knolls Research Laboratory, General Electric Company, Schenectady, N. Y., for his cooperation in the preparation of the electron micrographs of the polar filaments; Dr. J. L. Lowe, Department of Botany and Plant Pathology, New York State College of Forestry, Syracuse, N. Y., for the time he so willingly gave explaining the operation of photographic equipment. To Mr. Felix J. Czabator, the writer is thankful for the critical reading of the thesis.